The Use of the SAEM Algorithm in MONOLIX Software for Estimation of Population Pharmacokinetic-Pharmacodynamic-Viral Dynamics Parameters of Maraviroc in Asymptomatic HIV Subjects

Phylinda Chan, Philippe Jacqmin, Marc Lavielle, Lynn Mcfadyen, Barry Weatherley

The Use of the SAEM Algorithm in MONOLIX Software for Estimation of Population Pharmacokinetic-Pharmacodynamic-Viral Dynamics Parameters of Maraviroc in Asymptomatic HIV Subjects

Phylinda L. S. Chan PhD[1*], Philippe Jacqmin PhD[2], Marc Lavielle PhD[3], Lynn McFadyen PhD[1] and Barry Weatherley PhD[1]

[1] Global Pharmacometrics, Pfizer Primary Care Business Unit, Sandwich, UK.
[2] Exprimo NV, Mechelen, Belgium.
[3] INRIA Saclay, France.

[*]Submitting and corresponding author:
Phylinda L. S. Chan
Pfizer Ltd
Ramsgate Road,
Sandwich,
Kent CT13 9NJ
UK

Phone: 01304 644906
Fax: 01304 652590
E-mail: phylinda.chan@pfizer.com

Running head: Population PK-PD-viral dynamics analysis of maraviroc data using MONOLIX

Keywords: Maraviroc, MONOLIX, SAEM, Viral Dynamics, Population PKPD

Word count: 4374

Number of Tables: 2

Number of figures: 3

Summary (422 Words)

Previously NONMEM VI had been used to analyse rich maraviroc plasma concentration and viral load data arising from 63 asymptomatic HIV infected patients using a sequential approach for estimating parameters for a pharmacokinetic-pharmacodynamic-viral dynamics (PKPD-VD) model. The limitations are extensive computation times and convergence problems resulting from numerical difficulties in optimizing the linearized likelihood.

Using simulated viral load data for the given maraviroc monotherapy study design, the viability of NONMEM VI and MONOLIX version 2.4 to perform parameter estimation of the PKPD-VD model was assessed. Simulated data was also used to test if an effect compartment and/or a lag time could be distinguished to describe an observed delay in onset of viral inhibition using MONOLIX. The preferred model was then used to describe the observed maraviroc monotherapy data using MONOLIX. Parameter estimates obtained from three modelling approaches were compared; i) sequential PKPD-VD with fixed individual Empirical Bayesian Estimates (EBE) for PK, ii) sequential PKPD-VD with fixed population PK parameters, and iii) simultaneous PKPD-VD.

Using NONMEM VI many convergence problems (56%) were experienced with fitting the sequential PKPD-VD model to the simulated data. Comparing the sequential modelling approach with the two different software packages, MONOLIX took less time to generate population and individual estimates including diagnostics (default setting) than with NONMEM VI without diagnostics.

For the given maraviroc monotherapy study design, it was difficult to separate the viral dynamics system delay from the delay due to pharmacological effects. The preferred model included a lag time without inter-individual variability.

Parameter estimates from the MONOLIX analysis of observed data were comparable across the sequential methods with fixed individual EBEs or population PK parameters and with simultaneous PKPD modelling approaches. For the sequential method, computation time is approximately 25% less when fixing individual EBEs PK parameters and discarding the concentration data in the PD-VD parameter estimation step compared with fixed population PK parameters and retention of concentration data in the PD-VD parameter estimation step. No computation time was gained for the sequential method with fixed population PK parameters compared with the simultaneous PKPD-VD modelling approach.

The current analysis demonstrated that SAEM algorithm in MONOLIX is a useful tool for fitting complex mechanistic models requiring multiple differential equations. The implemented SAEM algorithm allowed simultaneous estimation of PKPD and viral dynamics parameters, as well as investigation of different model sub-components during the model building process which was not possible with NONMEM (version VI or below). MONOLIX provides a better alternative to NONMEM version VI or older when facing lengthy computation time or problems in convergence with complex models.

Introduction

Maraviroc (UK-427,857) is a reversible and selective antagonist of the human chemokine CCR5 receptor [1]. It has been approved for use in combination with other antiretroviral agents for the treatment of subjects infected with CCR5-tropic human immunodeficiency virus type 1 (HIV-1). Two short-term (10 days) monotherapy phase 2a studies (A4001007 and A4001015) were performed in asymptomatic CCR5-tropic HIV-1 infected subjects during the development program [2]. The maraviroc doses ranged from 25 mg once daily (QD) to 300 mg twice daily (BID). The mean HIV-1 viral load declined in a dose-dependent fashion with up to 1.6 $\log_{10}$ RNA copies/mL achieved (at day 11) with 300 mg BID [2].

Mathematical models have been widely used to describe the dynamics and interaction of target $CD4^+$ cells, actively, latently, persistently and defectively infected cells and plasma virus in HIV-1 infected asymptomatic subjects after initiation of antiretroviral therapy [3, 4, 5]. These viral dynamics models employ a set of differential equations to describe the viral dynamics. The maraviroc monotherapy data has previously been analyzed in a two-stage approach by fitting a pharmacokinetic-pharmacodynamic (PKPD) viral dynamics (VD) model [6,7] using nonlinear mixed-effects modelling for estimation of fixed effects, inter-individual and residual variability implemented in the NONMEM software [8]. NONMEM estimation methods include a first order method (FO) and first order conditional estimation (FOCE) method, both of which involve linearization of the regression function with respect to the random effects [9, 10, 11, 12]. Some of the practical drawbacks and/or limitations when performing

PKPD-VD parameter estimations with the FOCE method in older versions of NONMEM (VI and below) are

i) very long computation times;

ii) convergence problems resulting from numerical difficulties in optimizing the linearized likelihood;

iii) model instability necessitating a two-stage approach (PK modelling followed by PKPD-VD modelling) and limitation of number of parameters that can be estimated;

iv) difficulties with models that have change points e.g. lag times.

These factors make it very difficult to develop these complex PKPD models or to perform simultaneous PKPD-VD modelling with NONMEM (version VI and below).

MONOLIX [13] implements a stochastic approximation (SA) of the standard expectation maximization (EM) (=SAEM) algorithm for nonlinear mixed-effects models without approximations. The SAEM algorithm replaces the usual estimation step of EM by a stochastic procedure which has been shown to be very efficient with improved convergence toward the maximum likelihood estimates [14]. A review of population analysis methods and software using examples for complex pharmacokinetic and pharmacodynamic methods concludes that EM methods (performed by S-ADAPT, PDx-MCPEM and MONOLIX) have greater stability in analyzing complex PKPD models and can provide accurate results with sparse and rich data [12].

The objectives of the present analysis were to assess the SAEM functionality implemented in MONOLIX for complex mechanistic models in the application of

population PKPD-VD modelling of maraviroc monotherapy PK and viral load data. Three key questions are addressed:

i) The viability of NONMEM VI and MONOLIX to perform parameter estimation of a PKPD-VD population model using simulated data;

ii) Determination of a preferred model using MONOLIX looking at lag time versus an effect site delay model (ke0 model) using simulated data;

iii) Comparison of parameter estimates with sequential (two-stage) versus simultaneous modelling approaches using MONOLIX and observed data from studies A4001007 and A4001015.

The aim of this paper is thus not to directly compare parameter estimates from MONOLIX and NONMEM VI, but to explore an alternative tool that may allow more efficient/stable estimates of PKPD and viral dynamics parameters within a nonlinear mixed effects framework with two levels of random variability.

Methods

Data

An analysis of the data from two randomized, placebo-controlled, phase 2a monotherapy studies (A4001007 and A4001015) has been performed; rich plasma maraviroc concentrations (1250 samples) and viral load data (1169 observations) were available from 63 asymptomatic CCR5-tropic HIV-1 infected patients. Approval from local ethics committees was obtained and written informed consent was obtained from all subjects. These studies are described in more detail in [2].

Patients were randomly assigned to the following treatment groups: maraviroc 25 mg QD, 50, 100 or 300 mg BID, or placebo under fasted conditions in study A4001007;

maraviroc 150 mg BID (fed and fasted), 100 or 300 mg QD, or placebo under fasted conditions in study A4001015. In both studies, patients received treatment for 10 days and were followed up for 30 days after the last dose. The current analysis included data arising from patients who were assigned to one of the maraviroc treatment arms and excluded placebo data.

Blood samples were collected for determination of maraviroc plasma concentrations pre-morning dose on days 1-10, and at specified times up to at least 24 hours post dose on day 10. In A4001007, several additional samples up to 12 hours post-morning dose on day 1, as well as 48, 72 and 120 hours post dose on day 10 were collected. Plasma samples were analysed to determine maraviroc concentrations using solid phase extraction followed by liquid chromatography with tandem mass spectrometric detection. The same analytical method was used in both studies. The lower limit of quantification was 0.5 ng ml^{-1}. Plasma HIV-1 RNA viral load were assessed at screening, randomization, and on days 1-13, 15, 19, 22, 25 and 40 using the Roche Amplicor v1.5 RT-PCR assay (Roche Diagnostics). Baseline viral load was computed as the mean of the three $\log_{10}$ transformed predose values.

PKPD-Viral Dynamics Model

The PKPD-VD model has 3 components [7]:

i) a population PK model describing the PK of maraviroc;

ii) a PD model describing the inhibition of infectivity rate;

iii) a disease model describing the viral dynamics in HIV-1 infected patients.

Two levels of random effects are included, inter-individual and residual variability.

PK Model

A basic 2 compartment disposition model, parameterized as clearances (total CL and intercomparmental Q) and volumes (central V2 and peripheral V3), with first-order absorption (ka), an absorption lag time (LagC), food effects on ka and F1 (relative bioavailability) and an additive residual error model was used to fit the log-transformed maraviroc concentrations. The individual Empirical Bayesian Estimates (EBEs) of PK parameters were used to predict the drug concentration at the specific times when viral loads were measured and these were then used as input data to the second stage sequential PKPD-VD modelling.

PD Model

Based on the known mechanism of action of maraviroc, the effect was modelled using an inhibitory Emax model acting on the infection rate constant of the virus and target cells. An area of interest in modelling HIV-1 viral load changes in response to treatment is exploration of the observed time delay component of response to drug treatment.

After the start of antiretroviral therapy, a delay (1-2 days) in the effect on viral load is usually observed regardless of the antiretroviral agent (including its PK and/or its class) [15, 16, 17, 18]. The cause of this delay (see Figure 1 for maraviroc) is probably multi-factorial, i.e. time required for drug absorption, disposition, interaction with the target receptor/enzyme, activation of subsequent cellular or intra-cellular pathways, as well as the clearance of free virus particles and the death of infected cells. Different modelling approaches can be used to describe such a delay, e.g.

i) introduction of a lag time between PK and PD;

ii) the use of an effect compartment model to describe the time lag between plasma drug concentration and drug effect with an equilibration half time parameter (ke0) [19];

iii) an onset/offset model where different rate constants are allowed for the onset and offset of response.

All of the above approaches increase the complexity of the model and make estimation with NONMEM (version VI and below) more difficult. This offered a further opportunity for testing MONOLIX functionality.

In previous analyses of the maraviroc monotherapy data using NONMEM VI (unpublished data), the delay in onset and offset of viral inhibition was modelled with a lag time (LagE) and an effect compartment model with an equilibration rate constant (ke0). This allows the shift of the PK profile resulting in equilibrium between PK and onset of viral inhibition.

Viral Dynamics Model

Details of the viral dynamics model are described elsewhere [3, 4, 5, 6, 7]. Briefly, the dynamics and interaction of target $CD4^+$ cells, actively infected cells, latently infected cells and viruses in HIV-1 infected asymptomatic patients after initiation of antiretroviral therapy were modelled with a set of differential equations (Equation 1).

Target cell (activated CD4 + cells) : **Equation 1**

$$\frac{dT}{dt} = b - d_1 \cdot T - (1 - INH) \cdot i \cdot V \cdot T$$

Actively Infected cell (*s*hort - lived) :

$$\frac{dA}{dt} = f_1 \cdot (1 - INH) \cdot i \cdot V \cdot T - d_2 \cdot A + a \cdot L$$

Latently Infected resting cells (long - lived) :

$$\frac{dL}{dt} = f_2 \cdot (1 - INH) \cdot i \cdot V \cdot T - d_3 \cdot L - a \cdot L$$

Infectious Virus (copies HIV - 1 RNA):

$$\frac{dV}{dt} = p \cdot A - C \cdot V$$

where b is the activation rate constant of healthy target cells (T); d_1 is the death rate constant of T cells; i is the infection rate constant of T cells; V is the number of virus particles; f_1 is the fraction of healthy T cells which become short-lived infected T cells (A); d_2 is the death rate constant of short-lived infected T cells; $f_2 = (1-f_1)$ is the fraction of healthy T cells which become latently infected resting cells (L); d_3 is the death rate constant of latently infected resting cells; a is the reactivation rate constant of latently infected resting cells; p is the viral production rate constant of short-lived infected T cells; c is the death rate constant of virus. The persistently and defectively infected cells with a very long half-life were excluded in the current analysis as they are not relevant to short-term (10 days) data.

The *in vivo* maraviroc IC_{50} and viral dynamics parameters (basic reproductive ratio (RR0), activation rate of uninfected cells (b) and death rate of actively infected cells (d_2)) were estimated. Remaining viral dynamic inputs were fixed to values based on literature ranges.

The basic reproductive ratio (RR0) is a derived model parameter computed as the ratio of the birth rate constants to the death rate constants (Equation 2). RR0 gives the average number of offspring generated by a single virus particle in the absence of constraints [4]. When RR0 in the presence of an inhibitor is below 1, the system theoretically goes to extinction. When RR0 is above 1, the system adjusts to the inhibition and re-equilibrates to a new steady state.

$$RR0 = \frac{b}{d_1} \cdot i \cdot \frac{p}{c} \cdot \left(\frac{f_1}{d_2} + \frac{f_2 \cdot a}{d_2 \cdot (d_3 + a)} \right) \qquad \textbf{Equation 2}$$

The reproduction minimum inhibitory concentration (RMIC) is defined as the concentration of an antiretroviral compound that decreases RR0 to below the break point of 1 which results in eradication of the disease [20]. Given that RR0 and IC_{50} are highly correlated during the estimation process, RMIC was computed (Equation 3) to make more appropriate comparisons of these key parameter estimates obtained from different models or algorithms in the current analysis.

$$RMIC = (RR0 - 1) \cdot IC_{50} \qquad \textbf{Equation 3}$$

Software

The current analyses were performed using:

i) A nonlinear mixed-effects modelling methodology as implemented in the NONMEM software system, version VI level 1.2 [8] (patched with updated subroutines according to instructions provided from GloboMax/ICON on 30 August 2007). NM-TRAN subroutines version IV level 1.1, and the PREDPP model library (ADVAN6 TOL=5), version V level 1.0 utilizing Intel-based PC Workstations running Red Hat Linux (3.4.6-8) operating system (GRID system) and GNU Fortran

compiler (GCC 3.4.6 20060404) were used. Parameter estimation was performed using the FOCE method with interaction.

ii) The SAEM algorithm as implemented in MONOLIX version 2.4 via MATLAB version 7.5.0 on a Microsoft Windows XP operating system installed on a ThinkPad T61 with Intel® Core™ Duo CPU T7300 processors @ 2.00 GHz and 1.96 GB of RAM.

Analysis Plan

To address the key questions, the current analysis consists of 3 parts as shown in the schematic analysis plan shown in Figure 2. These are discussed in detail below.

Part 1: Assessing Model Viability in NONMEM VI and MONOLIX with Simulated Data

The first question to be addressed was the viability of NONMEM VI and MONOLIX to perform parameter estimation for the PKPD-VD model. Simulation of 50 datasets of viral load profiles were performed in NONMEM VI using identical study designs to the maraviroc monotherapy studies (subject numbers, doses and observations) and parameter estimates from a previous PKPD-VD analysis of the data in NONMEM VI including inter-individual (on IC_{50}, RR0, b and d_2) and residual variability but not including parameter uncertainty (unpublished data, details in PKPD-Viral Dynamics Model section).

The viral inhibition was driven by the predicted PK profile based on the population PK parameter estimates excluding inter-individual and residual variability. This was done to eliminate the potential impact of maraviroc PK on the viral dynamics parameter estimation.

The simulated concentration and viral load data were then fitted separately using NONMEM VI (FOCE with interaction) and MONOLIX. Model viability was assessed by the number of runs that terminated with successful minimization and other termination messages in NONMEM VI, as well as the precision of parameter estimates (compared with the "true" values used in simulation) in NONMEM VI and MONOLIX.

If a NONMEM VI run terminated due to "rounding errors", one more attempt/run was made by using the final parameter estimates as the initial parameter estimates. If the second attempt was terminated due to minimization successful, the run was classified as successful, otherwise it was classified as run terminated with "rounding errors".

Part 2: Determination of Preferred Model in MONOLIX using Simulated Data

Since different modelling approaches can be used to describe the delay in onset and offset of viral inhibition, another key question was to determine the preferred model fit using MONOLIX for a given maraviroc monotherapy study design with simulated data. While the simulation PD model had both a lag time (LagE) and an effect compartment model (ke0) describing the delay in onset and offset of viral inhibition, the 50 simulated viral load datasets were fitted to different PD models using MONOLIX, i.e. inclusion/exclusion of LagE and an effect compartment, as well as with/without inter-individual variability on LagE and ke0.

The preferred model was selected based on the precision of parameter estimates, standard error of population parameters, correlation of estimates, log-likelihood (by

Monte-Carlo Importance Sampling), information criteria such as Akaike information criterion (AIC) and Bayesian information criterion (BIC).

Part 3: Comparison of Parameter Estimates Obtained from Sequential and Simultaneous Modelling Approaches using MONOLIX

Finally, the observed maraviroc concentration and viral load data were fitted with the preferred model (defined in Part 2) with 3 different modelling approaches using MONOLIX;

i) Sequential PK and PD-VD, where PK parameters were estimated from the PK data alone, then the PD-VD parameters were estimated based on the PD data and the fixed individual EBE PK parameters. PK data was discarded in the PD-VD parameter estimation step;

ii) Sequential PK and PD-VD with PK parameters fixed to population estimates obtained from a separate PK analysis. The PD-VD parameters were estimated conditional on the fitted PK model and also the PK data as concentration data were retained;

iii) Simultaneous PKPD-VD, where PK and PD-VD parameters were estimated simultaneously in the presence of concentration and viral load data.

The aim was to assess the performance of parameter estimation in the presence/absence of concentrations when coupling/uncoupling the PK and PD-VD components of the PKPD-VD model.

Results

Part 1: Assessing Model Viability in NONMEM VI and MONOLIX

When analyzing the 50 simulated viral load data sets with NONMEM VI, 22 runs experienced numerical difficulties, of which 18 runs did not execute a single iteration; 6 runs terminated with "rounding errors" (on the second attempt). Only 22 runs terminated with "minimization successful", of which half failed to run a covariance step. Of the 22 successful runs, 8 had unreasonable estimates of ke0 (4 runs with 131-171 fold increases, 2 runs with 11.7 and 1621 fold increases respectively, 2 runs with 2.7-5 fold decreases) and/or LagE (2 runs with 2-3 fold decreases). For the 2 runs with unreasonable estimates of both ke0 and LagE, ke0 (1.07 and 0.564 d^{-1}) was 2.7 and 5.1 fold lower than expected, while LagE (0.368 and 0.483 d) was >2 fold lower than the true parameter values (ke0 = 2.86 d^{-1}, LagE = 1.13 d).

As expected parameter estimates were obtained for all 50 simulated data sets using MONOLIX. Six runs had unreasonable estimates of ke0 (4 runs with 2 fold lower estimates), RR0 (1 run with 5.5 fold higher estimate) and/or IC_{50} (2 runs with 2.2 and 47 fold higher estimates) compared with the values used for simulation. For the run with both unreasonable estimates of RR0 and IC_{50}, IC_{50} (418 ng/mL) was 47-fold higher than the "true" parameter value of 8.66 ng mL^{-1}. For both NONMEM VI and MONOLIX, parameter estimation of ke0 was troublesome.

The computation time for the 22 successful NONMEM runs ranged from 1.75 to 384.7 hours (median ~ 3 hours) without any post processing for diagnostics. With MONOLIX, computation time was approximately 2 hours per run including

diagnostics using the default settings (number of simulation samples: visual predictive checks (VPC) = 100, normalized prediction distribution errors (NPDE) = 500, Monte-Carlo size used for estimating the log-likelihood (LLP) = 10000).

Part 2: Determination of Preferred Model using MONOLIX

A general finding when fitting the 50 simulated viral load data sets using MONOLIX with different PD-VD models was the difficulty in determining both ke0 and LagE parameters given the viral load sampling times. The preferred model was the lag time model without an effect compartment and without inter-individual variability on LagE. This model described 48 (96%) simulated data sets the best (Table 1) based on the criteria listed in methods. The run time for the PD-VD estimation step ranged from 1.5 to 2.5 hours.

The preferred model with LagE but without inter-individual variability on LagE was taken forward for Part 3 analysis.

Part 3: Comparison of Parameter Estimates Obtained from Sequential and Simultaneous Modelling Approaches using MONOLIX

The pharmacokinetic model parameter estimates obtained from sequential and simultaneous PKPD-VD modelling approaches using MONOLIX are presented in Table 2. Food effects on absorption rate constant (ka) and relative bioavailability (F1) were modelled as fractional change with fasted status as the reference group. Due to the limited amount of fed data and the potential distortion of the PK model by the mis-specified PD model, the impact of food effect on ka and F1 were very different between the sequential and simultaneous PKPD modelling approaches. However, the relative standard errors suggest that the food effect on ka, particularly for the

simultaneous PKPD analyses, were not precisely estimated. Nevertheless, the structural PK parameters and associated inter-individual variability were similar between the sequential and simultaneous PKPD modelling approaches with small relative standard error (RSE).

The PD-VD model parameter estimates, along with the computed parameter RMIC, obtained from the sequential and simultaneous PKPD approaches are presented in Table 2. The computed RMIC, the viral dynamics parameters and their associated inter-individual variability were comparable across the 3 different modelling approaches for the given drug effect (PD) model. For the sequential method with fixed individual EBE PK (discarding PK data in the PD-VD parameter estimation step), the computation time was approximately 25% less than the sequential method with fixed population PK parameters (PK data retained in the PD-VD parameter estimation step). Interestingly, no computation time was gained by fixing population PK parameters in the sequential method compared with the simultaneous PKPD-VD modelling approach as run times ranged from 10 to 16 hours including diagnostics using default settings. The goodness-of-fit plots for the final PKPD-VD model using a simultaneous PKPD modelling approach are presented in Figure 3.

Discussion

NONMEM is a widely used tool for population pharmacokinetic-pharmacodynamic modelling. However, as shown in the current analysis, when complex semi-mechanistic models involving the use of a differential equation solver are necessary, NONMEM (versions up to VI) often experiences convergence problems resulting from numerical difficulties in performing parameter estimates. In addition, the long

computation time and the model instability limit the use of NONMEM (versions VI and below) for model building with models such as this example of a combined PKPD-VD model. As modelling and simulation activities move towards more complex mechanistic models it becomes necessary to investigate more practical tools, with better estimation methods such as SAEM which are not available in NONMEM versions VI or earlier. Bauer et al [12] have published a review benchmarking commonly available population analysis tools. This review includes a summary of the statistical theory behind the methods with testing of 4 models including one example of more complex PKPD model requiring differential equations requiring numerical integration. At this time MONOLIX 2.4 was not available. However they conclude that EM methods have the advantage of greater stability in population analyses of complex PKPD models with reduced bias in assessing sparse and rich data than NONMEM FOCE [21].

The SAEM algorithm has previously been used by Lavielle and Mentre [22] to perform population pharmacokinetic analysis of a protease inhibitor, saquinavir, with large inter-individual variability. Samson et al. [23] have also successfully applied the SAEM algorithm to describe the longitudinal decrease in $\log_{10}$ viral load (left censored data) after initiation of antiretroviral treatments, with a right-truncated Gaussian distribution. In addition to the ability of performing parameter estimation, the relatively short computation time (compared with NONMEM VI) allows exploration of different components of PD models and different modelling approaches to assess the impact of PK data and model on PD parameter estimation, and vice versa.

NONMEM VI and MONOLIX were compared in the first part of this work using 50 simulated data sets. In this test NONMEM VI experienced numerical difficulties with 44% of the data sets. In addition a further 12% of runs finished with rounding errors and 22% had a failed covariance step. The SAEM algorithm implemented in MONOLIX on the other hand always produced estimates of PKPD-VD parameters using either a sequential or simultaneous modelling approach, although in a minority of cases these were not very likely. Because parameter estimates for fixed effects and variances as well as standard errors are generated in all runs with MONOLIX, the viability to perform PKPD-VD parameter estimation was determined by assessing the precision of parameter estimates as in relative standard error estimates. With NONMEM VI more difficulties in estimating both ke0 and LagE (with relatively little information available from study design) were experienced while unlikely parameter estimates for IC_{50} and RR0 compared with simulated model parameters were generated only occasionally (12%) using MONOLIX.

Using simulated data sets, the current analysis attempted to separate the system delay from the delay of pharmacological effect using a lag time and an effect compartment. Unfortunately, for the given maraviroc monotherapy study design, it was difficult to separate the PD lag due to drug effects from system delay. Herz et al. [24] developed a mathematical model which incorporated an intracellular phase of the viral life-cycle to account for the virus production lags. The authors of that study concluded that plasma virus data alone do not allow a clear distinction between the delays of pharmacological action, intracellular delays and the clearance of free virus during the transition phase. It was also suggested that frequent clinical measurements for 2 days after initiation of antiretroviral therapies are required in order to improve parameter

estimates. In addition, frequent clinical measurements after the termination of antiretroviral therapies also provide very valuable information. For the model parameterization in the current analysis, the key to improve the precision of basic reproductive ratio (RR0) is the clinical measurement of viral load in the rebound period upon termination of antiretroviral therapies. Thus it can be concluded that data or study design limitations rather than a limitation with the MONOLIX software are an issue in parameter identification.

With the PKPD-VD model with lag time only, the LagE estimate was approximately 1.5 day. This is consistent with the observed delay of viral load drop after the initiation and termination of antiviral therapy (Figure 1) and an approximate 2 day half-time of the free virus particles and/or virus producing cells [25, 26, 27].

For good PKPD modelling practice, one should always examine the correlation between the pharmacokinetics of a compound and the drug effects (surrogate biomarkers or clinical measurements), as well as their interactions, i.e. the impact of one on the other. The current analysis compared parameter estimates obtained from different modelling approaches, sequential methods with fixed individual EBEs or population PK parameters and a simultaneous method using MONOLIX. In general, with the maraviroc monotherapy study design and data, the structural parameters of the PK, PD and VD model components were comparable across different modelling approaches. Zhang et al. have demonstrated that PK fits using a simultaneous modelling approach can be quite sensitive to the PD model, particularly when there was misspecification in the PD model [28]. Interestingly, Zhang et al also found that with the First Order Conditional Estimation (FOCE) method in NONMEM, the

sequential modelling approach saved about 40% of computation time compared with the simultaneous modelling approach [29]. In the current analysis, with the SAEM algorithm implemented in MONOLIX, no computation time was gained by using the sequential PKPD modelling approach with fixed population PK parameters with retention of PK data, when compared with simultaneous PKPD modelling. However, approximately 25% of computation time was gained by using the sequential method with fixed individual EBE PK parameters and discarding of PK data in the PD-VD parameter estimation step. Computation time was not directly compared between NONMEM VI and MONOLIX because different systems (parallel GRID for NONMEM VI and desktop for MONOLIX) were used. Also much time was wasted with NONMEM VI runs that did not terminate successful. Due to the high failure rate and the long computation time, it was not practical to perform parameter estimation in NONMEM VI using the same hardware system as was used for MONOLIX.

Though the objective of the current analysis was not to directly compare parameter estimates from MONOLIX with previous NONMEM analyses, the consistency of the parameter estimate for IC_{50} provided confidence in the use of SAEM for this PKPD-VD model. It should be noted that the published NONMEM analysis using data from study A4001007 used equivalent constant concentration [7] instead of the individual predicted PK profile to drive viral inhibition.

In conclusion, the current analyses demonstrate that SAEM algorithm in MONOLIX is a useful tool for complex mechanistic models requiring multiple differential equations. The implemented SAEM algorithm allowed simultaneous estimation of PKPD and viral dynamics parameters, as well as investigation of different model sub-

components during the model building process which was not possible with NONMEM (version VI and older). Future testing could include the comparison of the SAEM algorithm between MONOLIX and NONMEM VII.

Acknowledgements

INRIA was paid by Pfizer Inc. for developing, implementing and testing the model in MONOLIX.

P.L.S.C. and L.M. are employees of Pfizer Ltd. B.W., and P.J. are paid consultants to Pfizer Global Research and Development, Sandwich, Kent, UK.

The authors thank Professor Mats Karlsson of Uppsala University for his advice during the analysis; Professor Karlsson was a paid consultant to Pfizer Global Research and Development, Sandwich, Kent, UK. The authors also thank Dr Raymond Miller for reviewing the draft manuscript.

Tables

Table 1 Selection of preferred model given the maraviroc monotherapy study design

Model	With Inter-individual variability on LagE and/or ke0?	Number of time selected as preferred model [%]
No lag time No effect compartment	No	0
Lag time only	No	48 [96]
Lag time only	Yes	4 [8][a]
Effect compartment only	No	0
Effect compartment only	Yes	0
With lag time and effect compartment	No	6 [12][b]
With lag time and effect compartment	Yes	0

[a] 2 out of 4 runs were indistinguishable from Lag time only model without ω[LagE]
[b] All 6 runs were indistinguishable from Lag time only model without ω[LagE]

Table 2 Comparison of PK, PD and VD parameter estimates obtained from sequential and simultaneous PKPD modeling approaches in MONOLIX

	Sequential PKPD with fixed individual EBEs PK parameters		Sequential PKPD with fixed population PK parameters		Simultaneous PKPD	
	Population estimate	RSE (%)	Population estimate	RSE (%)	Population estimate	RSE (%)
PK						
CL (L d^{-1})	5500	6	5500 FIX	-	5180	6
V2 (L)	274	12	274 FIX	-	349	10
Q (L d^{-1})	1140	8	1140 FIX	-	1290	8
V3 (L)	1040	9	1040 FIX	-	1130	9
Ka (d^{-1})	8.11	10	8.11 FIX	-	9.36	10
Food on Ka	-0.755[a]	36	-0.755 FIX	-	-0.141[c]	222
F1	1 FIX	-	1 FIX	-	1 FIX	-
Food on F1	-0.244[b]	45	-0.244 FIX	-	-0.476[d]	14
LagC (d)	0.0211	10	0.0211 FIX	-	0.0178	13
ω[CL] (%)	46.6	9	46.6 FIX	-	46.9	9
ω[V2] (%)	60.6	16	60.6 FIX	-	55.5	15
ω[Q] (%)	35.1	20	35.1 FIX	-	47.2	15
ω[V3] (%)	40.0	22	40.0 FIX	-	51.4	15
ω[Ka] (%)	64.1	11	64.1 FIX	-	69.9	11
ω[LagC] (%)	39.0	26	39.0 FIX	-	60.2	20
Additional Error (%)	45.4	2	45.4 FIX	-	44.6	2
PD-VD						
RR0	5.33	10	5.92	11	4.96	9
b [d^{-1}]	1.22	15	1.18	13	1.36	14
d_2 [d^{-1}]	0.797	4	0.755	3	0.841	3
IC_{50} (ng mL^{-1})	8.27	19	6.73	22	8.57	24
LagE (d)	1.52	1	1.35	3	1.43	6
ω[RR0] (%)	78.6	10	79.4	10	64	10
ω[b] (%)	114	9	103	9	110	9
ω[d_2] (%)	29.3	11	20.6	12	19.6	13
ω[IC_{50}] (%)	137	11	160	10	175	10
Additive Error (%)	47.9	2	48.1	2	47.9	2
RMIC (ng mL^{-1})	35.8	-	33.1	-	33.9	-
-2 x log-likelihood	-		2367		2390	
AIC	-		2387		2440	
BIC	-		2409		2493	

[a] p value = 0.0049
[b] p value = 0.028
[c] p value = 0.65
[d] p value < 0.0001
RMIC = Reproduction minimum inhibitory concentration: $(RR0-1)\cdot IC_{50}$

Figures

Figure 1 Mean change from baseline of HIV RNA (log_{10} copies mL^{-1}) over 10 days of maraviroc treatment

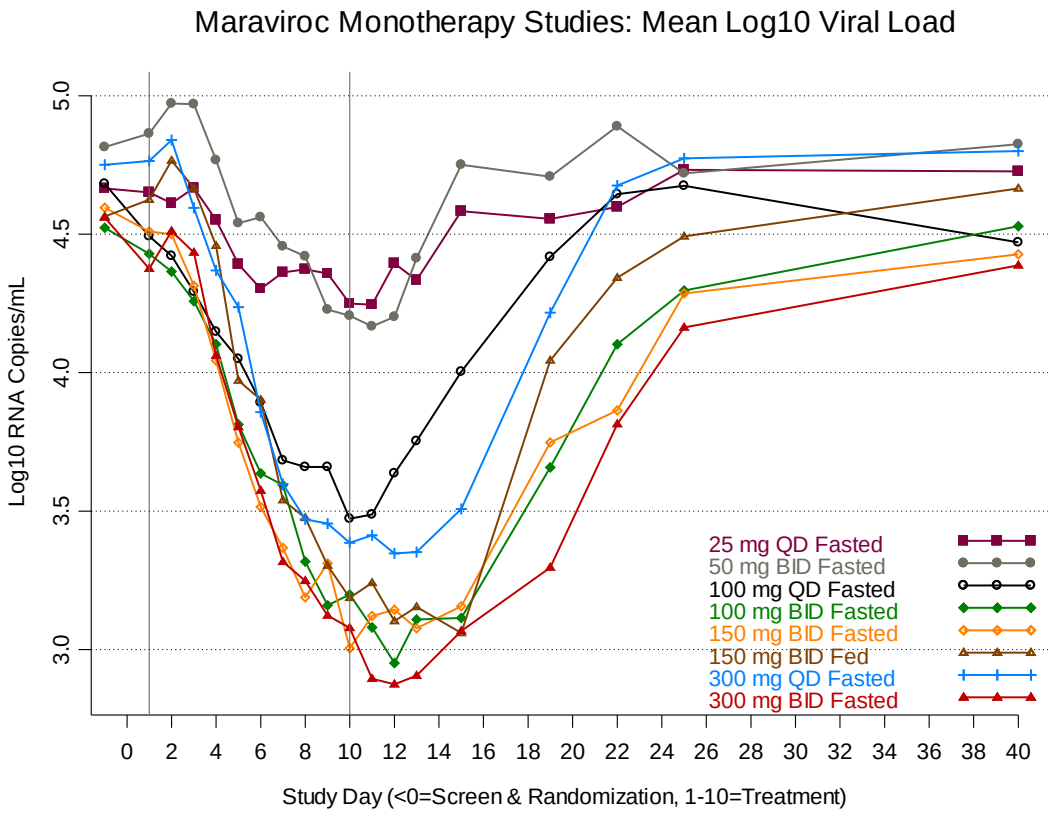


Figure 2 Schematic representation of the analysis plan

Simulation model = PKPD-VD with LagE and ke0,
IIV on IC_{50} and VD parameters (RR0, b and d_2)

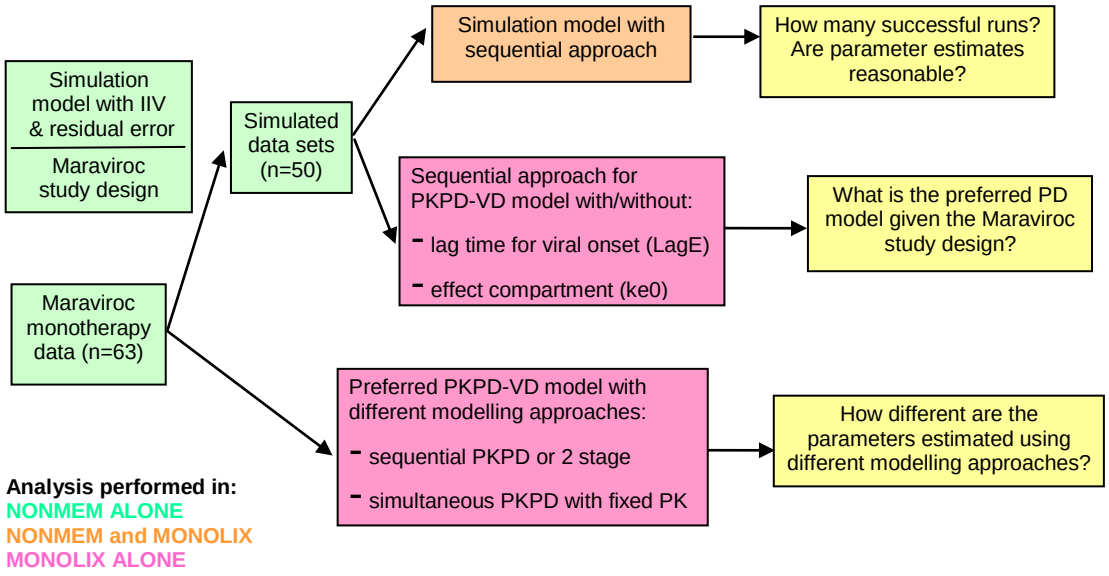
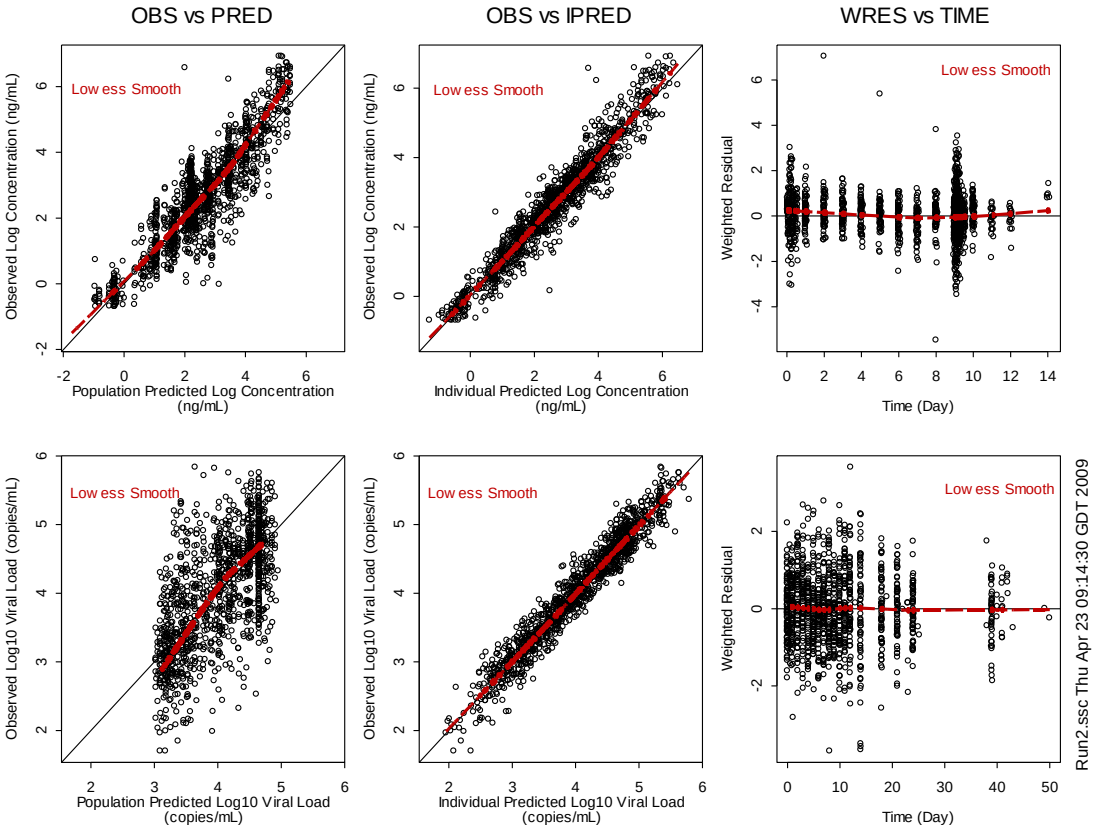


Figure 3 **Basic goodness of fit for the final PKPD-VD model using a simultaneous PKPD modeling approach. Top panel for maraviroc concentration; bottom panel for HIV RNA.**



References

1 Wood A, Armour D. The discovery of the CCR5 receptor antagonist, UK-427,857, a new agent for the treatment of HIV infection and AIDS. Prog Med Chem. 2005;43:239-271.

2 Fätkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, Hoepelman AIM, Saag MS, Goebel FD, Rockstroh JK, Dezube BJ, Jenkins TM, Medhurst C, Sullivan JF, Ridgway C, Abel S, James IT, Youle M, van der Ryst E. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1 Nat Med. 2005;11(11):1170-1172.

3 Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M, Ho DD. Decay characteristics of HIV-1-infected compartments during combination therapy Nature. 1997;387(6629):188-191.

4 Bonhoeffer S, May RM, Shaw GM, Nowak MA. Virus dynamics and drug therapy. Proc Natl Acad Sci U S A. 1997;94(13):6971-6976.

5 Funk GA, Fischer M, Joos B, Opravil M, Günthard HF, Ledergerber B, Bonhoeffer S. Quantification of in vivo replicative capacity of HIV-1 in different compartments of infected cells. J Acquir Immune Defic Syndr. 2001;26(5):397-404.

6 Rosario MC, Jacqmin P, Dorr P, van der Ryst E, Hitchcock C. A pharmacokinetic-pharmacodynamic disease model to predict in vivo antiviral activity of maraviroc Clin Pharmacol Ther. 2005;78(5):508-519.

7 Rosario MC, Poland B, Sullivan J, Westby M, van der Ryst E. A pharmacokinetic-pharmacodynamic model to optimize the phase IIa development program of maraviroc. J Acquir Immune Defic Syndr. 2006;42(2):183-191.

8 Beal SL, Sheiner LB. NONMEM Users Guides. Ellicott City, Maryland: Icon Development Solutions; 1988-1998.

9 Beal SL, Sheiner LB. Estimating population kinetics. Crit Rev Biomed Eng. 1982;8(3):195-222.

10 Lindstrom M, Bates D. Nonlinear mixed-effects models for repeated measure data. Biometrics. 1990;46:673-687.

11 Pandhard X, Samson A. Extension of the SAEM algorithm for nonlinear mixed models with 2 levels of random effects. Biostatistics 2009;10(1):121-135.

12 Bauer RJ, Guzy S, Ng C. A survey of population analysis methods and software for complex pharmacokinetic and pharmacodynamic models with examples. The AAPS Journal. 2007;9(1):E60-E83.

13 The MONOLIX software supported by the MONOLIX Group. http://www.monolix.org/

14 Kuhn E, Lavielle M. Maximum likelihood estimation in nonlinear mixed effects models. Comput Stat Data Anal. 2005;49:1020-1038.

15 Gruzdev B, Rakhmanova A, Doubovskaya E, Yakovlev A, Peeters M, Rinehart A, Dier Kd, Baede vDP, Parys W, Klooster Gvt. A randomized, double-blind, placebo-controlled trial of TMC125 as 7-day monotherapy in antiretroviral naive, HIV-1 infected subjects. AIDS. 2003;17(17):2487-2494.

16 DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, Elion R, Farthing C, Zhong L, Cheng AK, McColl D, Kearney BP, Corporate author: 183-0101 Study T. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment- experienced patients. J Acquir Immune Defic Syndr. 2006;43(1):1-5.

17 Schurmann D, Fatkenheuer G, Reynes J, Michelet C, Raffi F, van LJ, Caceres M, Keung A, Dunkle LM, et a, Corporate author UC, Corporate author UN, Corporate author S-P, Corporate author UK. Antiviral activity, pharmacokinetics and safety of vicriviroc, an oral CCR5 antagonist, during 14-day monotherapy in HIV-infected adults. AIDS. 2007;21(10):1293-1299.

18 Golden PL, Piscitelli S, Min S, Reddy S, Johns B, Garvey E, Ferris R, Hazen R, Edwards C, Woodward M, Yoshinaga T, Sato A, Iwashita S, Kadono K, Ohkawa T, Masuda K, Horie K, Fujiwara T, Palleja S. In vitro antiviral potency and preclinical pharmacokinetics of GSK364735 predict clinical efficacy in a phase 2a study. Poster #H-1047. The 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC); Chicago IL, United States; Sept 17-20, 2007.

19 Holford NH, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokinet. 1981;6(6):429-453.

20 Jacqmin P, McFadyen L, Wade JR. Basic PK/PD principles of proliferative and circular systems. Abstract #1194. 16th Meeting of the Population Approach Group in Europe; København, Denmark; 13-15 June, 2007.
21 Girard P, Mentré F. A comparison of estimation methods in nonlinear mixed effects models using a blind analysis. Abstract #834. 14th Meeting of the Population Approach Group in Europe; Pamplona, Spain; June 16-17, 2005.
22 Lavielle M, Mentré F. Estimation of population pharmacokinetic parameters of saquinavir in HIV patients with the MONOLIX software. J Pharmacokinet Pharmacodyn. 2007;34(2):229-249.
23 Samson A, Lavielle M, Mentré F. Extension of the SAEM algorithm to left-censored data in nonlinear mixed-effects model: Application to HIV dynamics model. Comput Stat Data Anal. 2006;51:1562-1574.
24 Herz AVM, Bonhoeffer S, Anderson RM, May RM, Nowak MA. Viral dynamics in vivo: limitations on estimates of intracellular delay and virus decay. Proc Natl Acad Sci USA. 1996;93(14):7247-7251.
25 Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, Saag MS, Shaw GM. Viral dynamics in human immunodeficiency virus type 1 infection. Nature. 1995;373(6510):117-122.
26 Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373(6510):123-126.
27 Nowak MA, Bonhoeffer S, Loveday C, Balfe P, Semple M, Kaye S, Tenantflowers M, Tedder R. HIV results in the frame: results confirmed. Nature. 1995;375(6528):193.
28 Zhang L, Beal SL, Sheiner LB. Simultaneous vs. sequential analysis for population PK/PD data II: robustness of methods. J Pharmacokinet Pharmacodyn. 2003;30(6):405-416.
29 Zhang L, Beal SL, Sheiner LB. Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. J Pharmacokinet Pharmacodyn. 2003;30(6):387-404.